

of this apparent amylase insufficiency remains unclear and further work is under way.

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## Four Mutations in the *SI* Gene Are Responsible for the Majority of Clinical Symptoms of CSID

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Intestinal symptoms in children are not unusual and often include abdominal bloating, vomiting, diarrhea, pain, and food aversion. If these symptoms persist, then physicians often will attribute them to food allergy, or in more persistent cases, will consider other disorders such as celiac disease, cystic fibrosis, or inflammatory bowel disease. Eventually, the consideration of congenital sucrose-isomaltase deficiency (CSID) will be entertained, and dietary restriction of complex carbohydrates and sucrose-containing beverages will be introduced (1). Confirmatory diagnosis of SI deficiency is dependent upon jejunal biopsy, with enzymatic measurements of lactase, sucrose, maltase, and isomaltase

(palatinase) (1). Patients with classic CSID will have normal lactase activity and a deficiency of sucrose and/or isomaltase.

With the advent of DNA technology, less invasive procedures for the confirmation of a genetic disease involves the detection of mutations within the causative gene. In the case of CSID, the gene *SI* is responsible for encoding the protein SI.

The phenotype of CSID is classically considered an autosomal-recessive condition that manifests in early childhood or infancy. Some heterozygotes have been confirmed to be symptomatic, but usually with less severe symptoms than homozygous relatives (2). Depending on the severity of the deficiency of sucrose and isomaltase, clinical symptoms can vary from severe, explosive diarrhea, dehydration, and growth failure to milder symptoms in adolescents or adults who may be clinically diagnosed as having dyspepsia or irritable bowel syndrome.

In individuals of European descent, the frequency of CSID is estimated at 0.2% to 0.05% (3,4) and is significantly higher in circumpolar populations in Greenland (5%–10%) and the Inuits of Alaska and Canada (3%–7%) (5,6).

Mutations within the *SI* gene are responsible for the phenotype of CSID. These mutations prevent the normal synthesis and transport of the protein responsible for sucrose (EC 3.2.1.48) and isomaltase (EC 3.2.1.10) and 80% of maltose digestion. The gene is located at chromosome 3q26.1, is approximately 100 kb in size, consists of 48 exons, and encodes a protein of 1827 amino acids. The isomaltase protein remains contiguous with the apical border of the villous cells, but the sucrose may be cleaved from the pro-SI by trypsin. The enzyme protein is anchored in the cytoplasm and cell membrane (amino acids 2–32) and has a short stalk region (amino acids 33–109) with isomaltase (amino acids 110–1007) and sucrose (amino acids 1008–1827) extending into the intestinal lumen (Fig. 1) (7).

## METHODS

To evaluate the genetic approach for the confirmatory diagnosis of CSID, we designed a study to obtain DNA samples from patients with a confirmed biopsy diagnosis. Families who volunteered for this study were recruited by 4 mechanisms: a letter to gastroenterologists, booth and literature at gastroenterology meetings, package inserts in Sucraid boxes, and notices on patient listservs by families participating in the study. The most successful mechanism was family contacts from the listserv. The above patient recruitment methods were approved by an institutional review board.

For inclusion in the study, probands had to have had a bowel biopsy with normal histology and normal lactase but a deficiency of sucrose and/or isomaltase, as defined by the testing laboratory. Twenty-seven children were deficient for both sucrose and isomaltase and 4 children were deficient for sucrose but had low normal activity for isomaltase. Biological samples for DNA isolation were obtained through saliva collected in Oragene-DNA self-collection kits (DNA Genotek Inc, Kanata, Canada) (8) or blood spotted onto filter paper. DNA from saliva was isolated according to the Oragene-DNA instructions provided from the kit, and DNA from blood spots was isolated using the 5-PRIME DNA isolation kit (ArchivePure DNA Blood Kits, 5-PRIME, Gaithersburg, MD). Primers to amplify 44 amplicons were designed to interrogate all 48 exons and 20 to 40 nucleotides of the flanking introns. The generated amplicons were evaluated for quality by first identifying the appropriate size on a 1% agarose gel and, if satisfactory, by subsequent DNA sequencing by Sanger dideoxy sequencing (9) using an ABI Prism 3130xl genetic analyzer (Applied Biosystems, Carlsbad, CA). The subsequent exons and flanking introns were compared to the GenBank number NM\_001041.3 to detect

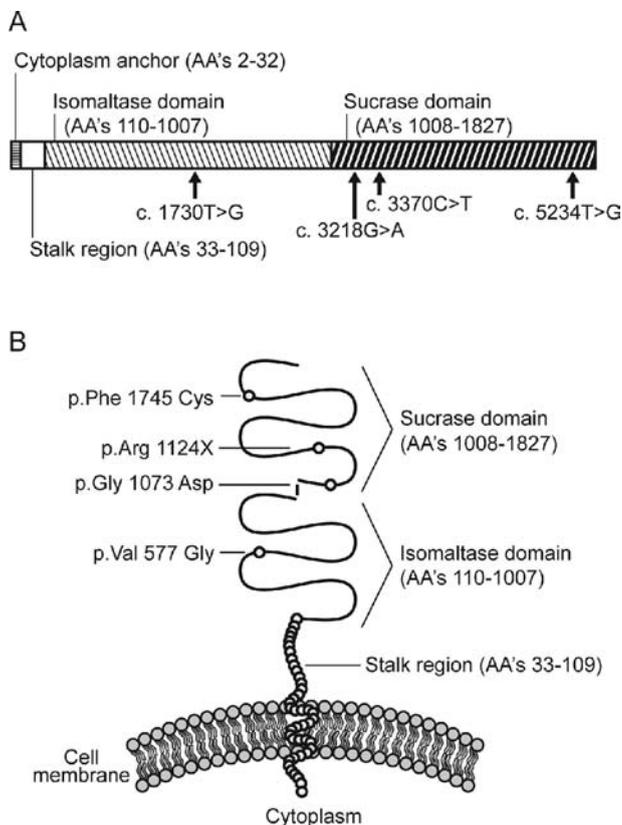
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Funding for the study was provided by QOL Medical.

The authors report no conflicts of interest.

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DOI: 10.1097/01.mpg.0000421408.65257.b5



**FIGURE 1.** A, Schematic representation of the gene structure for *SI* with the 4 most common nucleotide changes indicated in the regions coding for either isomaltase or sucrase. B, Topographic cartoon for the encoded protein for sucrase and isomaltase as it exists at the apical membrane of the villous brush border. The relative positions of the 4 common mutations are indicated.

nucleotide alterations. Nucleotide numbering used the A of the ATG translation initiation start site as nucleotide +1.

## RESULTS

From 31 probands identified to have CSID, a total of 56 abnormal alleles were identified. Twenty-five probands were homozygous or compound heterozygotes, and in 6 patients only a single nucleotide alteration was identified. One proband had no mutation identified. Thus, a total of 56 mutations were identified in this cohort of 31 patients. Twenty-two mutations were identified only once, 1 mutation was identified twice, and 4 mutations were responsible for the remaining 32 alterations. The most common mutation was c.3218G>A (p.Gly1073Asp) at a frequency of 0.31. The mutations c.1730T>G (p.Val577Gly), c.5234T>G (p.Phe1745Cys), and c.3370C>T (p.Arg1124X) had frequencies of 0.15, 0.07, and 0.06, respectively. Table 1 summarizes the frequency distributions.

Because patient selection was based on clinical symptoms with documented deficiency of sucrase and/or isomaltase from jejunal biopsies, each of the 4 mutations can be considered to be pathogenic. In addition, 3 of the 4 mutations (p.Val577Gly, p.Gly1073Asp, and p.Phe1745Cys) have been identified and studied previously for their cellular trafficking and have been confirmed to abolish sucrase and isomaltase activity (10). The

**TABLE 1.** Frequency distribution of *SI* gene mutations

Mutation	Exon	Domain	No. mutant alleles	Allele frequency
c.1730T>G p.Val577Gly	16	Isomaltase	8	0.14
c.3218G>A p.Gly1073Asp	27	Sucrase	17	0.31
c.3370C>T p.Arg1124X	28	Sucrase	3	0.05
c.5234T>G p.Phe1745Cys	46	Sucrase	5	0.09
Single mutations	1–26	Isomaltase	16	0.29
Single mutations	26–48	Sucrase	7	0.13

fourth mutation, p.Arg1124X, introduces a chain termination codon and interrupts the amino acid coding sequence.

Three of the 4 common mutations are in the sucrase domain, with 1 in the isomaltase domain. No mutations in this study were identified in the membrane anchor or stalk region. These 4 mutations account for 59% of the identified changes. The remaining 41% were rare events and are probably “private” mutations restricted to few individuals or families.

If one assumes Hardy-Weinberg equilibrium ( $p^2 + 2pq + q^2$ ) for the mutations in this population, there is an 83% probability that an individual of European ethnicity with CSID will have at least 1 of the 4 mutations ( $2pq + q^2$ ), where  $2pq = 0.48$  (1 common mutation) and  $q^2 = 0.35$  (2 common mutations). In 17% of severe cases ( $p^2$ ) of CSID, individuals will have 2 rare mutations not detected by a DNA diagnostic panel that assays only for the common 4.

Thus, consideration should be given to the use of genetic testing using saliva or blood for confirmation of CSID as an alternative to sedation, intestinal biopsy, and assay of intestinal enzymes. Such an approach would validate the diagnosis in a majority (83%) of symptomatic children of European ancestry with CSID.

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